Comparison of Fluorescein and Phycoerythrin Conjugates for Quantifying CD20 Expression on Normal and Leukemic B-Cells

For more than a decade, quantitative fluorescence calibration has been advocated for interlaboratory data comparisons and for the quality control of clinical flow cytometers. At the present, numerous fluorescence quantification methods have been developed and instrument calibration kits have been produced. However, due to the lack of follow-up support and consensus on resolving the differences with the use of these methods and materials, the quantification efforts have had a very limited clinical impact. NIST is enabling industry to overcome these technical hurdles by developing the measurement science for quantitative flow cytometry.

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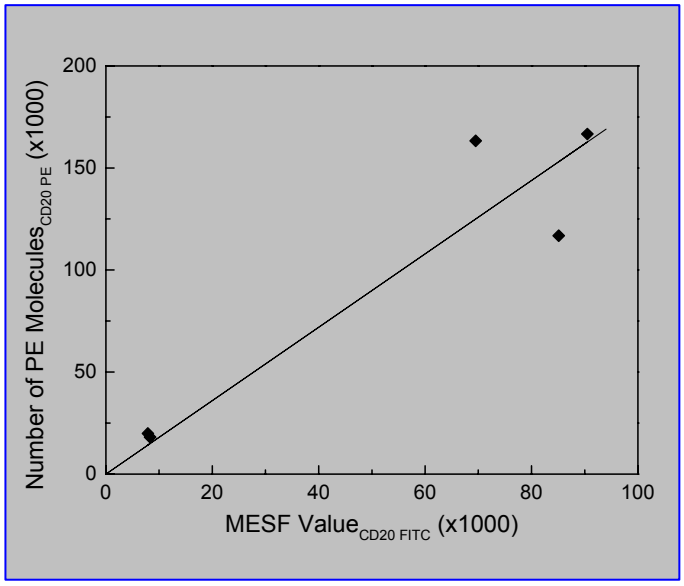
NIST researchers have quantified receptor expression on lymphocytes by using two different and yet widely used methods. The expression of the protein CD20 in B-cell chronic lymphocytic leukemia (B-CLL) is one of the best examples of such a biomarker. We measured CD20 expression on normal and B-CLL B-cells by using both fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated to the same monoclonal antibody (Mab). As a biological control and calibrator, the expression of the protein CD4 on T-cells was also quantified by using FITC and PE bound to Mab. Calibration with commercially available PE-labeled microspheres and the use of unimolar PE conjugates allowed for the direct measurement of the number of antibodies bound per cell (ABC) for both CD4 and CD20. Calibration of the FITC conjugates was based on the number of molecules of equivalent soluble fluorochrome (MESF) as determined with the use of NIST Reference Material 8640 microsphere standards. The measured MESF values were then converted to ABC values by using the CD4 T-cell as a biologic calibrator to normalize FITC and PE results for CD20 expression.

Thus, we have demonstrated that CD4 expression on T-cells can be used as a biological calibrator to quantify the number of antibodies bound per cell for CD20-FITC and show reasonable agreement between the two conjugates and two different fluorochromes. Issues regarding the accuracy of MESF microsphere calibrators and effective fluorochrome per protein ratios for FITC conjugates will require additional laboratory studies.



The figure shows the correlation between MESF values measured using CD20 FITC antibodies and the number of PE molecules determined for unimolar CD20 PE conjugates.